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Hyperfibrinolysis in a Case of Myelodysplastic Syndrome With Leukemic Spread of Mast Cells

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Mast cells (MC) are multipotent hemopoietic effector cells producing diverse mediators like histamine, heparin, or tissue type plasminogen activator. We report a 75-year-old male patient with myelodysplastic syndrome (MDS) of recent onset (3 months' history) associated with a massive leukemic spread of immature tryptase$^+$ MC (tentative term: myelomastocytic leukemia). The patient presented with pancytopenia, bleeding, hypofibrinogenemia, and an increased cellular tryptase level. Moreover, an excessive elevation of plasmin-antiplasmin complexes (9,200 ng/ml; normal range: 10–150), an elevated D-dimer, and an increase in thrombin-antithrombin III complexes were found. The identity of the circulating MC was confirmed by immunophenotyping (CD117/c-kit$^+$, CD123/IL-3Rα^-, CD11b/C3biR$^-$), biochemical analysis (cellular ratio [ng:ng] of tryptase to histamine >1), and electron microscopy. Bone marrow (bm) examination showed trilineage dysplasia (17% blasts), 30% diffusely scattered MC, and a complex karyotype. No dense, compact MC infiltrates (mastocytosis) were detectable in bm sections. Despite hyperfibrinolysis and mediator syndrome (flushing, headache), the patient received remission induction polychemotherapy (DAV) followed by two cycles of consolidation with intermediate dose ARA-C (2 × 1 g/m^2/day on days 1, 3, and 5). He entered complete remission after the first chemotherapy cycle without evidence of recurring MDS. Moreover, in response to chemotherapy, the hyperfibrinolysis and mediator syndrome resolved, and the circulating c-kit$^+$ MC disappeared. We suggest consideration of polychemotherapy as a therapeutic option in patients with high-risk MDS of recent onset, even in the case of MC lineage involvement. Am. J. Hematol. 61:66–77, 1999. © 1999 Wiley-Liss, Inc.

Key words: mast cells; myelodysplastic syndromes; mastocytosis; fibrinolysis; tryptase

INTRODUCTION

The myelodysplastic syndromes (MDS) comprise a heterogeneous group of clonal myelogenous disorders characterized by abnormal differentiation and maturation, bone marrow (bm) failure with cytopenia(s), and a genetic instability that is associated with a high risk of transformation into overt (secondary) leukemia [1–5]. The MDS are categorized according to: 1. their pathogenesis (primary MDS or MDS following a mutagenic event); 2. morphology of bm and peripheral blood (pb) cells; 3. special karyotypes; and 4. differentiation of dis-

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tinct cells, i.e., monocytes. The most useful and widely applied classification system is the proposal of the French-American-British (FAB) Cooperative Study Group [6,7]. This classification system was based primarily on morphologic criteria. However, among patients in one particular "FAB category," the prognosis and clinical picture may vary depending on the genes that underwent deregulation, cell type(s) involved, and the specific biologic properties of the clone(s).

Mast cells (MC) are multifunctional hemopoietic cells that develop from uncommitted $CD34^+$ progenitors. MC express a unique composition of antigens and can be distinguished from basophils and all other types of hemopoietic cells by their biochemical and functional properties [8,9]. Unlike basophils and other myeloid cells, MC produce significant amounts of tryptase and heparin [10,11] as well as enzymatically active uncomplexed tissue type plasminogen activator (tPA) [12,13]. MC also differ from other myeloid cells in surface receptor expression and functional responses to growth factors [14]. For example, mast cell growth factor (MGF), also termed stem cell factor (SCF), is a unique regulator of growth of human MC [15,16].

Involvement and differentiation of MC in MDS have been described for a subset of patients [17–20]. Such patients with MDS may have increased amounts of diffusely scattered MC in their bm without visible signs of mastocytosis (no compact dense MC infiltrates) [17]. In a few cases, however, a concomitant mastocytosis (with dense compact MC infiltrates) may be detectable [18–20]. In a very small subset of MDS patients (FAB group RAEB = refractory anemia with excess of blasts) an excessive leukemic spread of MC has been described [21]. These patients were found to have a complex karyotype, increased numbers of circulating c-kit^+, $tryptase^+$ MC, and a high cellular tryptase/histamine ratio (T/H) (>1 at an ng:ng basis) [21]. This unique type of MDS has tentatively been termed "myelomastocytic leukemia." In this study, we report another case of MDS with excessive leukemic spread of MC. In this particular case, a severe coagulation disorder with signs of hyperfibrinolysis and disseminated intravascular coagulation (DIC) was present. Because of rapid development and progression of the disease, and despite the unfavorable clinical situation, we decided to start a remission-induction polychemotherapy in this patient.

CASE REPORT

In March 1997, a 75-year-old male patient was presented because of bleeding from a palmar surgical wound, pancytopenia, and presence of atypical metachromatic cells (MCS) on a blood smear. He had a case history of renal cell carcinoma (resection of right kidney in 1978) and basal cell carcinoma (removed by surgery in 1986), but had not been exposed to mutagens or radiation. He had never suffered from a bleeding tendency, flushes, infectious diseases, or gastrointestinal ulcer. In 1996, he developed paresthesias and palmar stiffness caused by "Dupuytren contracture." A blood examination on November 28, 1996 showed a hemoglobin concentration of 13.8 g/dl, white blood cell count (WBC) of 5.4×10^9/L, and a platelet count of 141×10^9/L. A surgical correction of the palmar contraction site was performed on February 27, 1997. On the next day, he was found to bleed from the surgical wound with massive hematoma formation. Blood examination revealed pancytopenia with a hemoglobin of 7.7 g/dl, WBC 3.1×10^9/L, and platelet count of 24×10^9/L. The differential count showed 20% neutrophils, 29% lymphocytes, 3% monocytes, 3% eosinophils, and 45% atypical MCS. The activated partial thromboplastin time (aPTT) was 31.0 sec (normal: 20–40 sec), and the prothrombin time was 73% (normal: 70–120%). After platelet and erythrocyte transfusion, the "hematoma mass" was removed by surgery, but the bleeding could not be stopped. The patient was transferred to our department. At admission, he was still bleeding from the palmar wound and showed multiple hematomas and petechiae. No hepatosplenomegaly, lymphadenopathy, or other abnormalities were found on physical examination. The hemoglobin was 9.5 g/dl, WBC 3.6×10^9/L, and platelet count 61×10^9/L. The differential count showed 20% atypical MCS, 16% segmented neutrophils, 7% band forms, 5% eosinophils, 43% lymphocytes, 1% monocytes, 4% metamyelocytes, 3% myelocytes, and 1% blasts. The reticulocyte count was 46.6×10^9/L. The serum lactic dehydrogenase (LDH) amounted to 275 U/L. All other routine chemical laboratory parameters were normal. However, analysis of coagulation parameters revealed signs of DIC and hyperfibrinolysis, with a decreased serum fibrinogen (113 mg/dl), increase in D-dimer (33,725 ng/ml), increase in thrombin-antithrombin (TAT) complexes (78.4 ng/ml), increase in prothrombin fragments F1,F2 (32.2 nmol/L), and an excessive elevation of plasmin-antiplasmin complexes (9,200 ng/ml). The aPTT amounted to 39.5 sec. The bm smear showed trilineage dysplasia, with an increase in blasts (17%) and increase of atypical MCS (30%). The histology showed an elevation of $CD34^+$ blasts (10%) and a diffuse infiltration with small- to medium-sized immature MCS with round or bilobed nuclei. These cells were found to react strongly with antibodies against tryptase and c-kit (CD117). As assessed by combined toluidine blue/immunofluorescence staining, the majority of the MCS in the bm and pb expressed c-kit, whereas only few MCS (approximately 10%) stained positive for IL-3Rα (CD123). The diagnosis of MDS subtype RAEB (FAB criteria) with differentiation and leukemic spread of MC (tentative term: myelomastocytic leukemia) was established. The bm cells were found to

exhibit a complex karyotype. Because of the unfavorable prognosis (high-risk group according to International Prognostic Scoring System criteria [22]), remission induction polychemotherapy with the "DAV 3+5+7" protocol (45 mg/m^2/day daunorubicin days 1–3; 100 mg/m^2/day etoposide days 1–5; 2 × 100 mg/m^2/day cytarabine days 1–7) was started (despite the clinical situation and coagulation disorder). From day 4 after chemotherapy was started, the clinical situation worsened. The patient developed severe skin flushes, nausea, vomiting, and abdominal pain as well as bleeding, i.e., hemoptysis, melena, and new petechiae. Therapy included clotting factors, platelet concentrates, antihistamines, corticosteroids, and antibiotics. On day 12, the patient developed septicemia (temperature: 39.3°C) with pneumonia (right lower lobe) caused by *Pseudomonas aeruginosa* infection. Because of concomitant cardiac failure and transient atrial fibrillation, he was transferred to the intensive care unit. During the next few days the hyperfibrinolysis resolved and the septicemia was treated successfully with antibiotics. After hematological reconstitution (starting from day 19), the clinical situation improved rapidly. To shorten the time of aplasia the patient received granulocyte colony-stimulating factor (G-CSF) (480 μg/day s.c.) from day 14 to day 21. A bm examination on May 2, 1997 revealed complete remission (2% blasts). On May 30, 1997, the patient was discharged in good health and complete hematologic (trilineage) reconstitution. During the following weeks he received two cycles of consolidation therapy with intermediate dose cytarabine (ID-ARA-C) (2 × 1 g/m^2/day cytarabine on days 1, 3, and 5). No recurrence of MDS or hyperfibrinolysis was noted, and the c-kit$^+$ MC disappeared. On August 17, 1997, however, he died suddenly at home from cardiac arrest of unexplained reason.

REAGENTS, ANTIBODIES

RPMI 1640 medium was purchased from PAA (Linz, Austria); Iscove's modified Dulbecco's medium (IMDM), L-glutamine, penicillin, streptomycin, fungizone, and gentamycin from Gibco Life Technologies (Gaithersburg, MD); fetal calf serum (FCS) from Hyclone (Logan, UT); FITC goat F(ab')$_2$ antimouse IgG (H+L) from Caltag Laboratories (San Francisco, CA); and AB serum from SeraLab (Crawley Down, UK). The mAbs G3 (antitryptase) and B7 (antichymase) were from Chemicon (Temecula, CA); mAbs BEAR 1 (CD11b), QBEND10 (CD34), 84H10 (CD54), and SZ.21 (CD61) from Immunotech (Marseille, France); MEM-74 (CD17) from Monosan (Uden, Netherlands); W17/1 (CD88) from Serotec (Oxford, UK); III 204 (CD35), YB5.B8 (CD117), and 7G3 (CD123) from Pharmingen (San Diego, CA); and MMA (CD15) from Becton Dickinson (San Jose, CA). The mAb 1A2.C5 (CD117) was kindly provided by Dr. H.J. Bühring (Department of Internal Medicine II, University of Tübingen, Germany). The following mAbs were obtained from the Vth International Workshop on Human Leukocyte Differentiation Antigens (Boston 1993): K20 (CD29); L10 (CD43); A3D8 (CD44); CBR-IC3/1 (CD50); and 13C2 (CD51). A polyclonal rabbit antihuman myeloperoxidase (MPO) antibody was purchased from Dako (Glostrup, Denmark); a biotin-labeled horse antimouse antibody and the "ABC-Elite kit" from Vector Laboratories (Burlingame, CA); and a biotinylated goat antimouse antibody and peroxidase-conjugated streptavidin from Biogenex Laboratories (San Ramon, CA). Naphtol-ASD-chloroacetate, protease type XXIV and AEC (3-amino-9-ethylcarbazole) were from Sigma (St. Louis, MO); and Tris-hydroxymethyl-aminomethane from Biomol (Hamburg, Germany).

METHODS

Preparation and Culture of Cells

In our patient with MDS, mononuclear cells (MNC) were isolated from the pb (serial examinations starting before chemotherapy) and from bm aspirates (taken from the iliac crest before and after remission-induction chemotherapy) after informed consent was given. For determination of tryptase and histamine in blood MNC, pb was also obtained from seven healthy volunteers and one patient with chronic myeloid leukemia (CML); informed consent was obtained in each case. MNC were prepared by using Ficoll. The MC leukemia line HMC-1 [23] was kindly provided by Dr. J.H. Butterfield (Mayo Clinic, Rochester, MN). HMC-1 cells were cultured in IMDM with 10% FCS and antibiotics at 37°C and 5% CO_2. Primary MC were enriched from a surgical lung specimen (one patient with bronchogenic carcinoma; informed consent was given before surgery) by collagenase digestion and elutriation as described [24,25]. MC in the lung cell dispersate amounted to 14%.

In Vitro Exposure of Neoplastic Cells to Drugs

To analyze the in vitro response of the neoplastic cells to various cytotoxic drugs, bm and pb MNC (2 × 10^6 per well) were incubated with different concentrations of doxorubicin (10 and 100 μg/ml), fludarabine (5, 25, and 100 μg/ml), vincristine (100, 500, and 1,000 ng/ml), etoposide (18 and 60 μg/ml), or control medium, in six-well culture plates (Costar, Cambridge, MA) for 14 hr at 37°C/5% CO_2. After incubation, cells were collected and washed.

Electron Microscopy

Electron microscopy (EM) was performed on pb MNC and bm MNC obtained before chemotherapy as well as on pb MNC on day 4 after chemotherapy. In addition, bm

MNC and pb MNC (obtained before chemotherapy) were cultured in RPMI 1640 medium with 10% FCS and antibiotics in the presence or absence of drugs (doxorubicin, 10 μg/ml; fludarabine, 25 or 100 μg/ml; vincristine, 100 or 500 ng/ml; etoposide, 18 or 60 μg/ml) for 14 hr before analysis by EM. Preparation of cells and EM analysis was performed according to published techniques [26,27]. In brief, 2×10^6 MNC were collected and washed in PBS containing Ca^{2+} and Mg^{2+}. Cells were fixed in 1% paraformaldehyde, 1.25% glutaraldehyde, and 0.025% $CaCl_2$ buffered in 0.1 M sodium cacodylate (pH 7.4) at room temperature (RT) for 60 min. The cells were washed three times in 0.1 M sodium cacodylate buffer, suspended in 2% agar, and centrifuged. The pellets were postfixed with 0.66 M collidine-buffered 1.3% OsO_4, and stained en bloc with 2% uranyl acetate in sodium maleate buffer (pH 4.4) for 2 hr at RT. Pellets were then rinsed, dehydrated in an alcohol series, and embedded in EPON 812. Ultrathin sections were cut, placed on copper grids, and contrasted in uranyl acetate and lead citrate. Grids were viewed in a JEOL 1200 transmission electron microscope (Tokyo, Japan).

DNA Analysis

After incubation in control medium or drugs, cells were lysed in 5 mM Tris (pH 7.4), 1% SDS, and 5 mM EDTA. Lysates were incubated at 37°C in 50 μg/ml of RNase A (Boehringer Mannheim, Mannheim, Germany) for 2 hr, and consecutively with proteinase K (50 μg/ml) (Boehringer Mannheim) for 16 hr. DNA was isolated using phenol/chloroform isoamyl alcohol and precipitated in absolute ethanol and sodium acetate at −20°C overnight [27]. Thereafter, the DNA precipitate from each sample was dissolved in 20 μl Tris EDTA buffer (10 mM Tris-HCl and 1 mM EDTA, pH 7.4). Then, loading buffer (0.25% bromphenol blue, 0.25% xylene cyanol, 40% sucrose) was added (10% of total volume) before samples were transferred onto a 2% agarose gel (agarose was dissolved in 0.089 M Tris-borate, 0.089 M boric acid, and 0.002 M EDTA). The DNA gel electrophoresis was run at 75 volts for 3 hr.

Indirect Immunofluorescence Staining Technique

Expression of cell surface molecules on metachromatic cells (bm and pb) was analyzed by a combined toluidine blue/indirect immunofluorescence (IF) staining technique as described previously [24,28]. Briefly, cells were incubated with mAbs for 30 min (4°C), washed, and then incubated with fluorescein-conjugated "second-step" goat antimouse antibody. Cells were then fixed in glutaraldehyde (0.025%) for 1 min, washed, and stained with toluidine blue (0.0125%) for 10 min at RT. Cells were examined under bright field and fluorescence light by microscope.

Cytochemistry and Immunohistochemistry

Bm biopsy (iliac crest) specimens were fixed in formalin, decalcified in EDTA, and embedded in paraffin. Sections of 2 μm were cut, dewaxed in xylene, and treated with 0.3% methanol-H_2O_2 (30 min at RT). In case of antigen-unmasking using microwave (CD34 and MPO), sections were rehydrated through graded ethanol, and treated with 0.3% H_2O_2 in 0.05 M Tris-buffered saline (TBS) (pH 7.5). After each step, sections were rinsed twice in TBS. For several markers, sections were pretreated to enhance immunoreactivity [29,30]: For detection of CD117 and chymase, enzymatic digestion was performed with 0.05% protease type XXIV (5 min, 37°C); in the case of CD34 and MPO, sections were heated two times (5 min) by microwave oven; in the case of CD15 and tryptase, sections were not pretreated. Immunohistochemistry was performed using the avidin-biotin-immunoperoxidase staining technique as described [29–31]. Antibody binding was made visible by using AEC. Cells were counterstained in Mayer's Hämalaun. Expression of antigens in tryptase$^+$ MC was analyzed in serial bm sections. For evaluation of antigen expression in suspended MNC, cells were spun onto cytospin slides, fixed in acetone, and analyzed by indirect immunoalkaline phosphatase staining technique. Cytochemical analysis included toluidine blue- and Giemsa-staining as well as staining for chloroacetate esterase (CAE) using naphthol-ASD-chloroacetate as substrate.

Cytogenetic Analysis

Karyotyping was performed on Giemsa-banded metaphases from short-term cultures (24 to 27 hr) of unstimulated bm MNC as described [32]. Karyotypes were classified and are described according to the International System for Human Cytogenetic Nomenclature (ISCN) [33]. Fluorescence in situ hybridization (FISH) was performed on bm MNC using "whole chromosome painting probes" for 12 different chromosomes as described [34].

Analysis of c-kit for Activating Point Mutations

Sequence analysis of the c-kit kinase domain was performed on bm MNC and pb MNC from our patient. The mast cell line HMC-1, which exhibits c-kit point mutations at codons 816 and 560 of c-kit [35] served as positive control. Isolation of total RNA, cDNA synthesis, polymerase chain reaction (PCR) amplification, and direct nucleotide sequence analyses (from codon 537 to codon 596 and codon 783 to 844) were performed as described [36]. The sequences of the primers used for PCR analysis of codons 816 and 820 were: c-kit-1: 5′-CCCTAGACTTAGAAGACTTGCTGA-3′ and c-kit-2: 5′-AAAAATCCCATAGGACCAGACGTC-3′. Primers used for amplification of a cDNA fragment containing codon 560 of c-kit were: c-kit-3: 5′-GGTAACA

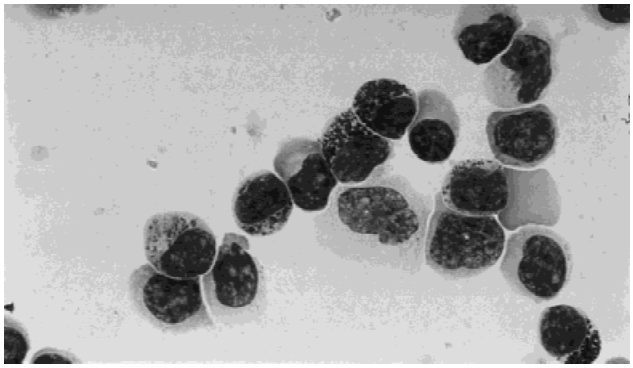

Fig. 1. Detection of atypical circulating MCS. Pb mononuclear cells were spun onto cytospin slide and stained with Giemsa. Note immaturity (blast-like morphology) of some of the MCS. As assessed by morphology it was impossible to decide whether MCS are MC or basophils.

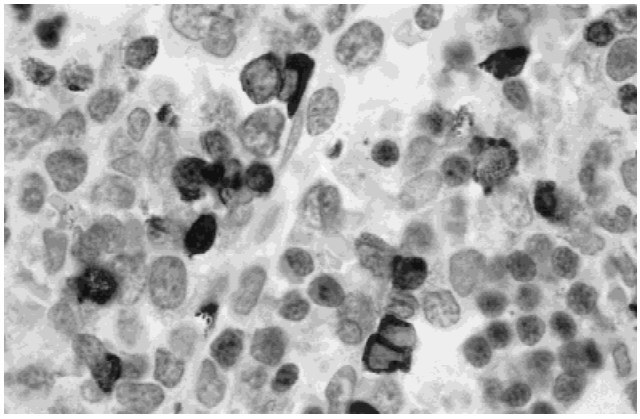

Fig. 2. Detection of MC tryptase in a bm biopsy section. A bm biopsy section was obtained before chemotherapy. The section was incubated with antitryptase mAb G3, and the reaction of the mAb with neoplastic MC in the bm made visible by avidin-biotin-immunoperoxidase staining technique (for technical details see text). Note immaturity and granular staining pattern of MC.

ACAAAGAGCAAATCCATCC-3′ and c-kit-4: 5′-TTGAGCATCTTTACAGCGACAGTCAT-3′. Point mutation (A→T) in position 2468 of codon 816 was also analyzed by restriction enzyme digestion as described by Nagata et al. [37].

Measurement of Tryptase and Histamine

Histamine and tryptase were measured in lysates of pb MNC obtained from our patient with MDS, one patient with CML, seven healthy controls, as well as HMC-1 cells. Histamine was measured by a commercial radioimmunoassay (Immunotech) as described [21]. Tryptase concentrations were measured by a fluoro-enzyme-immunoassay [FIA] (Pharmacia, Uppsala, Sweden) as described [38]. The detection limit of this assay was found to be 1 ng/ml. No cross-reactivity with histamine, heparin, or various cytokines was found.

Determination of DIC Parameters and Markers of Fibrinolysis

The serum concentrations of D-dimer, TAT complexes, and prothrombin fragments F1,2 were measured by standard immunoassays (Boehringer Mannheim). Plasma fibrinogen levels were determined according to the method of Clauss. Plasma tPA-, uPA-, and PAI-1 protein concentrations as well as plasmin-antiplasmin (PAP) complex concentrations were determined by commercial assays (Technoclone, Vienna, Austria).

RESULTS

Cytologic Examination of bm and pb Cells

At initial diagnosis, bm smears showed trilineage dysplasia with hypogranulated neutrophils and low numbers of megakaryocytes. An increase in blast cells (17%) was found. These blasts were medium sized with prominent nuclei containing one or two nucleoli. Auer rods were absent. The most intriguing finding was an increase of atypical metachromatically granulated cells (MCS) (30%). These MCS were small to medium sized, showed a pale cytoplasm, and round or bilobed nuclei. The cytoplasm contained few or multiple basophilic granules. Most cells appeared to be very immature, some of them resembling blasts that contained a few metachromatic granules. Typical (mature) tissue MC were not detectable. In blood smears, many atypical MCS (20–45%) were found (Fig. 1) with a similar morphology as compared with the bm. However, a few of these cells contained multilobed nuclei with a condensed chromatin suggesting the presence of basophils. In response to chemotherapy, both the blasts and atypical MCS decreased in number. After the first cycle, only a few atypical MCS were found in pb smears, and after the first consolidation, these cells disappeared. Also, the myelodysplastic bm was replaced by normal-appearing bm with complete trilineage reconstitution. No recurrence of blasts or atypical MCS was noted during the observation period.

Histology and Immunohistochemistry

Bm biopsy sections showed marked trilineage dysplasia and an increase in immature myeloid cells. Using an antibody against CD34, approximately 10% of all nucleated cells were found to be blasts. In addition, a marked diffuse infiltration of the bm with atypical immature MCS was seen. These cells reacted with mAbs against c-kit and tryptase (Fig. 2) suggesting the presence of MC. The calculated percentage of tryptase$^+$ MC in the bm was 30%. Compact dense tryptase$^+$ MC infiltrates were not detected. As assessed by serial section staining, the tryptase$^+$ MC did not react with mAb against chymase (MC_T). Cytospin staining confirmed expression of tryptase and

revealed expression of tPA in a subset of pb and bm cells. A summary of immunohistochemical staining results is depicted in Table I. After the first chemotherapy cycle the diffuse infiltrate of tryptase$^+$ cells had almost disappeared, and the number of CD34$^+$ cells was below 5%.

EM of Neoplastic Cells

EM confirmed the presence of immature atypical MC in both the bm and pb at the time of diagnosis. These cells were medium sized with multiple surface projections, and showed monolobed, bilobed, or even polylobed nuclei (Fig. 3). In most of these cells, the nuclei contained one or two nucleoli. In the cytoplasmic compartment, mitochondria as well as empty granule containers or granules partially filled with loose contents, were found. Granules containing scrolls or crystals could not be detected. In some immature MC, lipid bodies were found. Apart from the immature MC, many blasts and other immature myeloid cells were detected. Interestingly, we were able also to detect a smaller proportion of (immature) basophils. In vitro exposure of pb or bm MNC to etoposide or doxorubicin resulted in the appearance of many apoptotic cells. Induction of apoptosis by etoposide and doxorubicin was confirmed by DNA blotting (ladder-type fragmentation, not shown). Moreover, the in vitro induction of apoptosis could be confirmed by ex-vivo analysis of pb cells during chemotherapy. In particular, many of the pb MNC showed ultrastructural signs of apoptosis on day 4 after initiation of chemotherapy (not shown).

Surface Marker Analysis

To confirm further the identity of the metachromatic cells, surface marker analysis was performed on bm and pb MNC at initial presentation as well as during and after chemotherapy. At the initial presentation, a majority of the circulating metachromatic cells (85%) expressed c-kit (CD117). Interestingly, these cells were also found to react with mAbs against adhesion molecules including leukosialin (CD43), Pgp-1 (CD44), ICAM-3 (CD50), and ICAM-1 (CD54) as well as VLA-ß (CD29), vitronectin-receptor (VNR)α (CD51), and VNRß (CD61) (Table II.). By contrast, only a minority of the metachromatic cells expressed IL-3Rα (CD123), C3biRα (CD11b), lactosylceramide (CD17), CR1 (CD35), or C5aR (CD88) (Table II.). Corresponding data were obtained with metachromatic cells obtained from the bm. Thus, based on their surface marker profile, the majority of metachromatic cells resembled MC. During the chemotherapy phase, the c-kit$^+$ MC disappeared, whereas basophils, i.e., metachromatic cells expressing CD123/IL-3Rα, increased in number. Figure 4 shows the time course of the absolute number of c-kit$^+$ MCs. After the first chemotherapy cycle, the percentage of c-kit$^+$ MCs had decreased from 85% to 10% (of all metachromatic cells), and the absolute number of circulating c-kit$^+$ MCs from 764/μl to 106/μl pb. After the first consolidation, the percentage of circulating c-kit$^+$ MCs was <1%. These cells did not reappear in consecutive analyses (Fig. 4). After the first chemotherapy cycle, the percentage of basophils (CD123$^+$ metachromatic cells) in the blood increased to 90% of all metachromatic cells. After the second consolidation cycle, the percentage of CD123$^+$ MCs was 100% (of all metachromatic cells). At that time, almost all of the MCs in the peripheral blood were found to be mature basophil granulocytes by morphology (Giemsa staining).

Karyotype

Chromosomal analysis of bm MNC revealed a complex karyotype in 17 of 22 metaphases analyzed. In 16 of these 17 metaphases, the following karyotype was found using G-banding and FISH analysis: 44,X,–Y, r(2)(p13q13),dic(2;18)(p13;p11.2),der(4)del(4)(q21q31)ins(4;9)(q21;?),der(5)t(5;17;9)(q22;?;?),der(7)t(5;7)(?;p13),der(9)t(9;17)(q12;q21~23),dup(11)(q21q23),der(21)t(11;21)(?;p12). In one metaphase, a polyploid karyotype with 110 chromosomal elements was seen. In addition, one normal metaphase (46,XY) and four with loss of the Y chromosome (45,X,–Y) were detected. After the first chemotherapy cycle, 22 of the 35 analyzed metaphases showed a normal karyotype, and 13 displayed a loss of Y chromosome.

Analysis of c-kit Gene Sequences for Point Mutations

Recent data suggest that neoplastic cells in a subset of patients with mastocytosis including those who have additional hematologic abnormalities, exhibit point mutations in the c-kit kinase domain [37]. Therefore, we were interested to know whether our patient with MDS and leukemic spread of MC would exhibit such mutations. However, no mutations were found in the regions of the c-kit kinase domain analyzed (codons 537 to 596 and 783 to 844) in bm MNC. In the HMC-1 cell line, which served as control, the two known c-kit mutations in codons 816 (*Asp→Val*) and 560 (*Val→Gly*) were detectable.

Measurement of Tryptase and Histamine

Mast cells express significant amounts of tryptase and histamine, whereas basophils express significant amounts of histamine, but only trace amounts of tryptase. Therefore, the cellular T/H ratio is a useful marker to discriminate between MC and basophils (Table III.). Before chemotherapy, the T/H ratio in pb MNC in our patient with MDS amounted to 1.2 indicating the presence of MC (Table III.). However, in response to chemotherapy, the T/H ratio in the pb MNC decreased to 0.08 on day 45 (after chemotherapy was started). The calcu-

TABLE I. Expression of Leukocyte Antigens in Neoplastic MC in bm Sections*

CD/antigen	Ab	Ig	Source	Ab dilution	Reactivity of neoplastic MC	Reactivity of myeloid cells[a]
nc/tryptase	G3	IgG1	m	1:5,000	+	−
nc/chymase	B7	IgG1	m	1:1,500	−	−
nc/MPO	anti-MPO	Poly	r	1:400	−	+[a]
15/Lewis-x	MMA	IgM	m	1:20	−	+[a]
34/HPCA-1	QBEND10	IgG1	m	1:100	−	+[a]
117/c-kit	1A2.C5	IgG3	m	1:200	+	+

*MC, mast cells; bm, bone marrow; Ig, immunoglobulin; MDS, myelodysplastic syndrome; nc, not yet clustered; m, mouse; r, rabbit; HPCA-1, hemopoietic precursor cell antigen-1; Ab, antibody; poly, polyclonal; MPO, myeloperoxidase. In situ immunohistochemistry was performed in our patient with MDS at the time of diagnosis. The technique is described in the text. The neoplastic MC were found to react with a monoclonal Ab against tryptase (and an Ab against c-kit). Expression of other antigens in neoplastic MC was analyzed in serial bm sections.

[a]The Ab against MPO, Lewis-x, and HPCA-1 were found to react with (neoplastic) immature myeloid cells in the same bm sections. The antibody B7 against chymase was found to react with cutaneous MC (positive control, not shown).

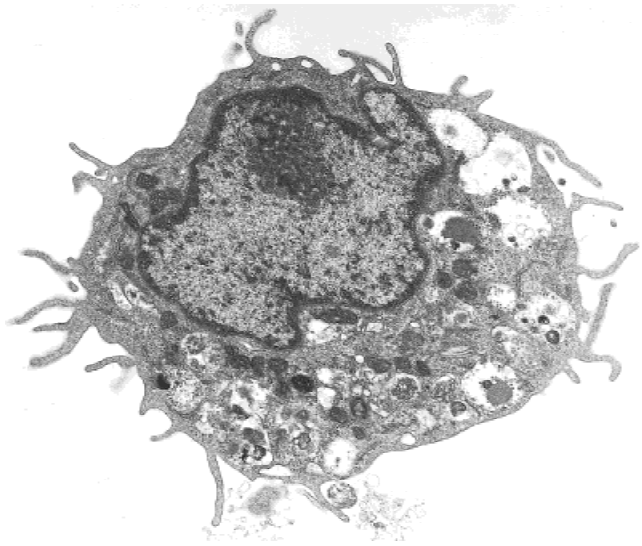

Fig. 3. EM; Pb and bm MNC were prepared for EM analysis as described in the text. Numerous circulating MC progenitors were detectable in the pb before chemotherapy. One typical immature MC is depicted. This cell shows numerous surface projections and a prominent nucleus with a large nucleolus. In the cytoplasm, some empty granules and some granules filled with small particles are seen. Magnification, ×10,000.

lated amount of tryptase per MCS was 0.55 pg before chemotherapy. After chemotherapy, the cellular level of tryptase decreased to 0.07 pg per MCS (day 45). The T/H ratio in lung MC was found to be 1.26 ± 0.26, whereas the T/H ratio in pb basophils (healthy subjects) was 0.05 ± 0.04. A summary of results is depicted in Table III.

Measurement of Coagulation- and Fibrinolysis-Related Parameters

At the initial presentation, a severe coagulation disorder with signs of massive hyper-fibrinolysis and DIC was detected (as major cause of bleeding tendency). In particular, we found a markedly decreased fibrinogen, an elevation of D-dimer, and elevated F1,2 and TAT complex concentrations. Moreover, an excessive elevation of serum PAP complexes was found (Fig. 5). Shortly after chemotherapy was started, the concentrations of D-dimer, F1,2, TAT complex, and PAP complex further increased. However, during the following days, the levels of D-dimer, F1,2, TAT, and PAP complexes declined, and after day 14 (when complete aplasia was reached), these parameters returned to normal values (Fig. 5). Interestingly, the plasma tPA concentration was normal before chemotherapy. However, the tPA concentration markedly increased after initiation of chemotherapy (Fig. 6). Then, the tPA levels declined, and returned to normal values until day 26. The plasma uPA and PAI-1 concentrations were normal during the whole observation period.

DISCUSSION

MDS represent a heterogeneous group of clonal myeloid disorders. In most patients, clinical symptoms arise from bm failure with occurrence of cytopenia(s) [1–3]. However, in some MDS patients the symptoms may also be caused by mediator production by clonal cells. We recently reported a massive leukemic spread of MC in patients with MDS [21]. In the current report, we describe a similar patient (MDS with leukemic mast cells) who suffered from massive hyperfibrinolysis and a mediator syndrome. In response to chemotherapy, the blasts and circulating MC disappeared, and the mediator syndrome (with hyperfibrinolysis) resolved. These data suggest that the products of clonal cells were critically involved in the coagulation disorder.

Although the coagulation disorder (hyperfibrinolysis) apparently was triggered by clonal (leukemic) cells, it

TABLE II. Expression of Surface Antigens on Circulating Metachromatic Cells*

CD	Antigen	mAb	Ig	Source	% reactive metachromatic cells	Phenotype of SMC	LMC	BA
CD11b	C3biRα	BEAR 1	IgG1	m	10%	−	−	+
CD17	LacCer	MEM-74	IgM	m	10%	−	−	+
CD29	β-chain, β1	K20	IgG2a	m	30%	+	+	+
CD35	CR1	III 204	IgG1	m	10%	−	−	+
CD43	Leukosialin	L10	IgG1	m	95%	+	+	+
CD44	Pgp-1	A3D8	IgG1	m	95%	+	+	+
CD50	ICAM-3	CBR-IC3/1	IgG1	m	95%	−	+	+
CD51	VNRα	13C2	IgG1	m	15%	±	±	−
CD54	ICAM-1	84H10	IgG1	m	60%	+	+	+
CD61	VNRβ	SZ.21	IgG1	m	20%	±	±	−
CD88	C5aR	W17/1	IgG1	m	05%	+	−	+
CD117	c-kit	YB5.B8	IgG1	m	85%	+	+	−
CD123	IL-3Rα	7G3	IgG2a	m	10%	−	−	+

*Expression of surface CD antigens on metachromatically granulated pb cells was analyzed at the time of diagnosis by using a combined toluidine blue/immunofluorescence staining technique (see text). The results are given as percentage of reactive cells (100 cells analyzed). A comparison with the well-established phenotype of lung mast cells (LMC), foreskin MC (SMC), and blood basophils (BA) is shown. The majority of circulating metachromatic cells in the patient's pb were found to express the mast cell phenotype, i.e., c-kit$^+$/IL-3Rα−/C3biRα−. Ig, immunoglobulin class; m, mouse; ICAM, intercellular adhesion molecule; MC, mast cells; LacCer, lactosylceramide. Data for LMC, SMC, and BA refer to published results [34]. VNR, vitronectin receptor.

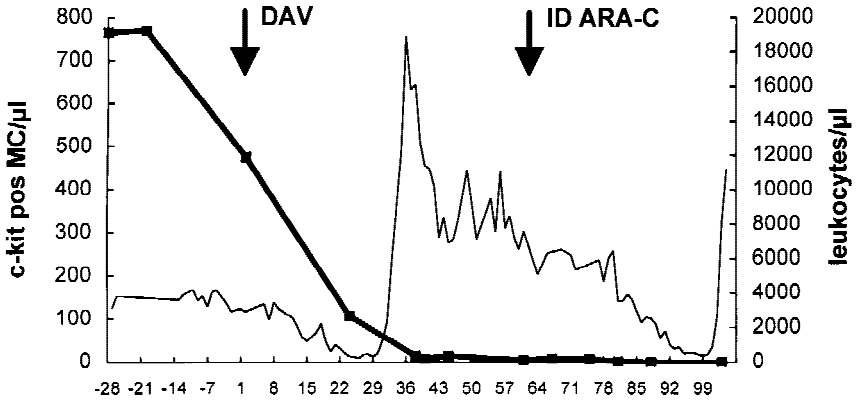

Fig. 4. Time course of the number of circulating c-kit$^+$ MC. The absolute numbers of c-kit$^+$ MC were calculated from WBC counts (shown as ——), differential blood counts, and percentages of c-kit$^+$ MCS determined by combined toluidine blue/IF double-staining technique (■–■). In response to chemotherapy, the number of c-kit$^+$ MC decreased. After the second consolidation, these cells even disappeared.

could not be specified whether the fibrinolysis was induced directly by these cells (primary hyperfibrinolysis), or occurred as a secondary event following DIC. The huge excess of PAP complexes over TAT complexes as well as detection of increased serum tPA (but not uPA) would suggest primary hyperfibrinolysis. In this regard it is noteworthy that normal MC as well as HMC-1 cells produce tPA, but do not express plasminogen activator inhibitors [12,13]. Moreover, the neoplastic cells in our patient reacted with anti-tPA antibody. However, apart from tPA, several other MC products may have contributed to the coagulation disorder. Thus, MC tryptase is able to degrade fibrinogen and activates prourokinase [39,40]. In addition, MC produce heparin [11]. Whether indeed and which MC products were involved in the coagulation disorder remains unknown.

Depending on the stage of cell maturation, environment (organ), and presence of distinct cytokines, MC express different patterns of enzymes [10,41]. MC tryptase supposedly is expressed at all maturation stages and all types of human MC [10,16,41]. By contrast, chymase expression is not detectable in very immature MC, but seems to be restricted to a subset of mature MC (MC_{TC}). In our patient, neoplastic MC were found to express tryptase, but did not express chymase. This lack of chymase is best explained by the immaturity of MC. In fact, neoplastic MC in patients with MC leukemia or malignant mastocytosis as well as HMC-1 all express tryptase, but lack significant amounts of chymase [42–44]. An alternative explanation for the lack of chymase would be MC heterogeneity. If so, the MC_T–, but not the (cutaneous) MC_{TC} type was involved in the neoplastic process. It is noteworthy that no cutaneous MC infiltrates were detectable in our patient, a phenomenon that also has been observed with patients suffering from true MC leukemia [45,46].

TABLE III. Measurement of Cellular Tryptase and Histamine*

Mediator	Patient's pbMCS DG	Patient's pbMCS CR	Normal donors pbMCS n = 7	CML pbMCS n = 1	HMC-1 n = 3	LMC n = 3
Tryptase, pg/cell	0.55	0.07	0.04 ± 0.03	0.02	0.19 ± 0.02	0.49 ± 0.15
Histamine, pg/cell	0.45	0.85	0.86 ± 0.28	1.16	0.42 ± 0.11	0.41 ± 0.19
T/H ratio	1.2	0.08	0.05 ± 0.04	0.02	0.47 ± 0.16	1.26 ± 0.26

*Cellular tryptase, cellular histamine, and the calculated cellular tryptase/histamine (T/H) ratio in the patient's pbMNC, in pbMNC of normal donors (n = 7), in pbMNC of a patient with CML (n = 1), in HMC-1 cells, and LMC (elutriated lung mast cells) were analyzed. In the patient with MDS, cellular tryptase was measured at the time of diagnosis (DG) as well as in complete remission (CR) on day 45 (after start of chemotherapy). Tryptase was measured by FIA, and histamine by RIA. Results represent the mean ± SD of seven donors, or the means ± SD of three experiments (HMC-1). As visible, a T/H ratio of >1 is associated with the mast cell lineage, whereas a T/H ratio substantially being <1, is indicative of the presence of basophils.

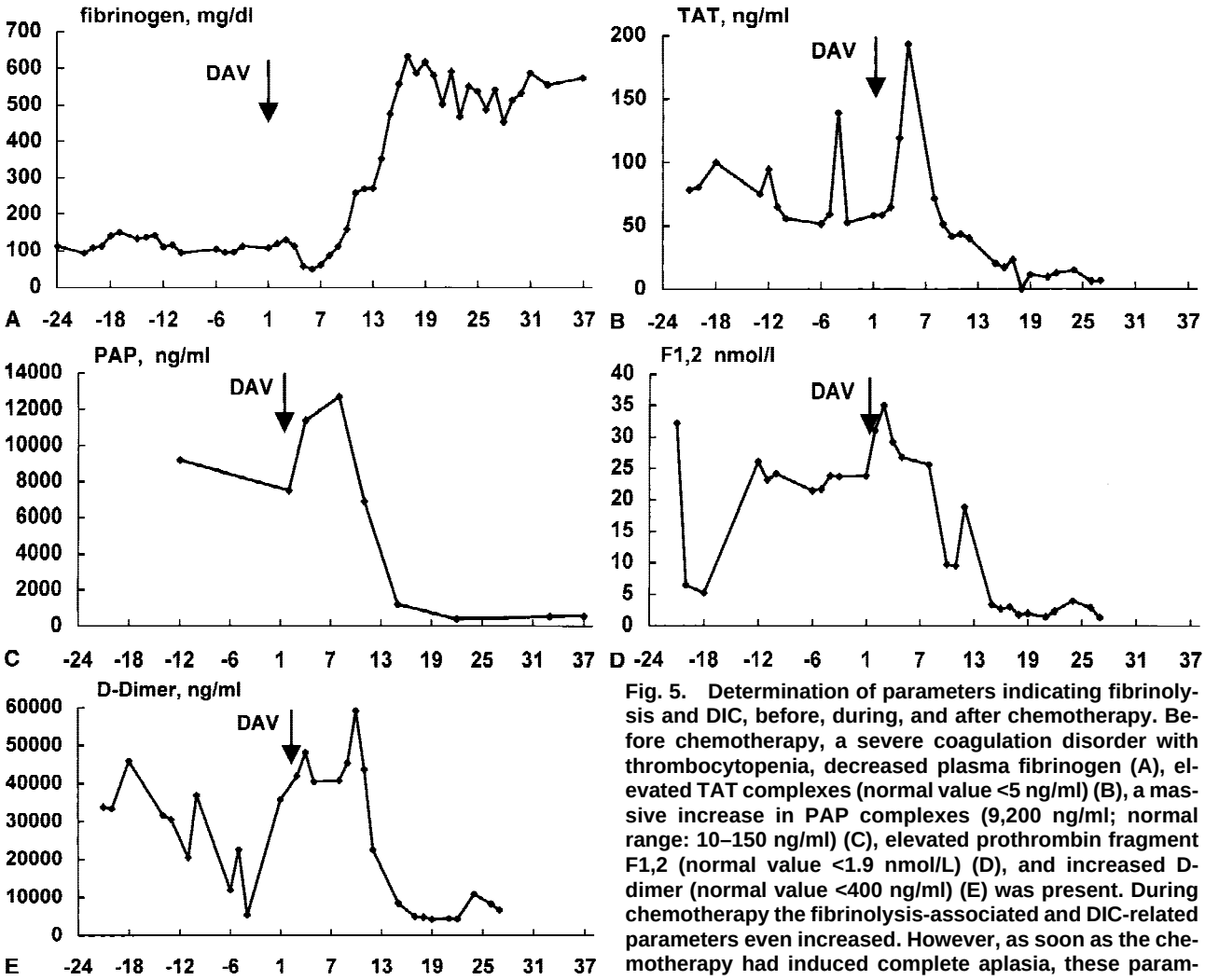

Fig. 5. Determination of parameters indicating fibrinolysis and DIC, before, during, and after chemotherapy. Before chemotherapy, a severe coagulation disorder with thrombocytopenia, decreased plasma fibrinogen (A), elevated TAT complexes (normal value <5 ng/ml) (B), a massive increase in PAP complexes (9,200 ng/ml; normal range: 10–150 ng/ml) (C), elevated prothrombin fragment F1,2 (normal value <1.9 nmol/L) (D), and increased D-dimer (normal value <400 ng/ml) (E) was present. During chemotherapy the fibrinolysis-associated and DIC-related parameters even increased. However, as soon as the chemotherapy had induced complete aplasia, these parameters declined, and when complete remission had been reached, most of them had returned to normal (or near normal) values.

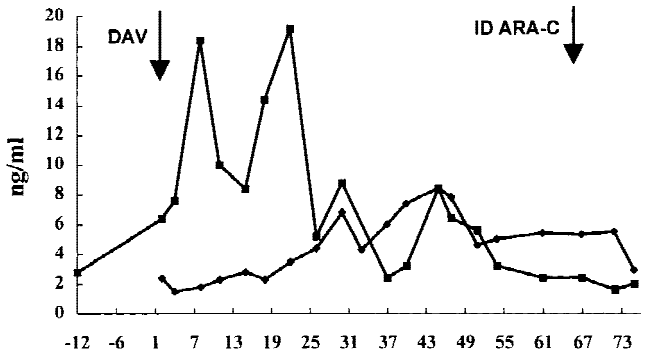

Fig. 6. Evaluation of plasma tPA and uPA protein concentrations. Before chemotherapy, the plasma tPA protein concentration was in the normal range (=2–8 ng/ml). However, in response to the chemotherapy, the plasma tPA concentration (■–■) increased. During the following weeks, the tPA level returned to the normal range. The uPA levels (♦–♦) remained within the normal range during the observation period.

Little is known so far about the pathophysiology underlying MC leukemia or MDS associated with a leukemic spread of MC. In the case of systemic mastocytosis, activating point mutations in the "kinase-domain" of c-kit (SCF receptor) have been described [37,47]. Such mutations also have been detected in the MC leukemia line HMC-1 [35] and may be responsible for ligand (SCF) independent kinase activity of c-kit and autonomous MC growth. However, in our patient, no c-kit mutations were detectable.

Another important question was why MC (usually fixed to the tissues) were spread into the circulation. One possible explanation would be cell immaturity. In fact, the normal immature MC progenitor is thought to be a circulating cell capable of entering the tissue by homing before terminal differentiation and maturation occurs [48,49]. A second explanation would be that distinct cell surface adhesion receptors, usually expressed by MC, were absent. In the present study we have analyzed expression of various adhesion-related molecules. However, we were not able to detect significant differences when the adhesion receptor profile of neoplastic MC was compared with the well-established phenotype of normal MC [14,28].

Patients with "MC proliferative disorders" may suffer not only from MC infiltrations in diverse organs, but also from a significant mediator syndrome [45,50]. In our patient, severe signs of mediator activity were also detectable. These symptoms included headache, nausea, and flushing. Interestingly, the symptoms were moderate before chemotherapy, but were severe after the start of chemotherapy. This may be explained by additional release of mediators induced by the chemotherapy. Administration of antihistamines, corticosteroids, platelets, and clotting factors lead to an improvement of mediator-related symptoms. The treatment decision (chemotherapy) in our case deserves special consideration. Thus, a number of facts argued against treatment with polychemotherapy. First, this patient was in a bad clinical situation, advanced age, and showed a complex karyotype. In addition, the involvement of MC was judged as a bad predictor, because MC leukemias usually do not respond well to polychemotherapy [46]. On the other hand, this patient had recent-onset MDS (normal blood picture 3 months before admission), no mutagenic event in his case history, normal cardiac function, and apparently a high risk to transform to AML according to a currently available scoring system (IPSS) [22]. In addition, the clinical situation did not improve after a short observation period. Ultimately, we decided to start a remission-induction chemotherapy. The good response to this chemotherapy was somehow unexpected, although we were able to demonstrate an in vitro response of neoplastic cells to various cytotoxic drugs. However, we certainly cannot predict (from a single case) whether other patients with similar disease may show a response to a polychemotherapy.

MC and basophils share a number of antigens and functional properties, but usually can be distinguished from each other by their morphology. However, in the case of immature neoplastic cells, it may be very difficult or even impossible to decide whether MCS belong to either the MC or basophil lineage by morphologic means alone [21,51]. Also MC in patients with MC disease may show an abnormal phenotype [52,53]. Therefore, recent investigations have focused on the phenotyping of MC in patients with MC proliferative disorders, using various staining techniques [21,43,52,53]. In the present study, we have investigated the phenotype of the circulating MCS in our patient to confirm MC-lineage involvement. In these experiments, the MCS expressed the MC phenotype in surface marker (c-kit$^+$, IL-3Rα^-) and biochemical analyses (high cellular tryptase; T/H ratio >1). In addition, the MCS were found to be MC by EM analysis. Interestingly, MC exhibited a monolobed or multilobed nucleus, as has been described for immature cultured [51,54] or neoplastic [21,42,54] MC.

A number of studies have reported the involvement and differentiation of MC in MDS [17–21]. A subset of patients may have increased amounts of diffusely scattered MC in their bm without signs of mastocytosis [17]. In certain cases, however, a concomitant mastocytosis is detectable [18–21]. In our patient, no dense MC infiltrates were detectable in the bm, excluding the possibility of an associated (underlying) mastocytosis. Rather, this patient had an excess of diffusely infiltrating MC in his bm, as well as an excessive leukemic spread of MC. We recently have described a similar MC spread in two other MDS patients [21]. We now propose the tentative term "myelomastocytic leukemia" for such cases. This term

was selected to separate the disease from mastocytosis and true (primary) MC leukemia. Moreover, we felt that this disorder should be regarded as an MDS entity similar to chronic myelomonocytic leukemia. The alternative term "secondary mast cell leukemia" was also considered. However, this possibly would lead to confusion, because the term "secondary" in the MDS classification, relates to a preceding mutagenic event.

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